Clinical Nutrition xxx (xxxx) xxx

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Randomized Control Trials

The effects of vitamin D and probiotic co-supplementation on glucose homeostasis, inflammation, oxidative stress and pregnancy outcomes in gestational diabetes: A randomized, double-blind, placebo-controlled trial

Mehri Jamilian [a], Elaheh Amirani [b], Zatollah Asemi [b, *]

[a] Endocrinology and Metabolism Research Center, Department of Gynecology and Obstetrics, School of Medicine, Arak University of Medical Sciences, Arak, Iran

[b] Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, Iran

ARTICLE INFO

Article history:
Received 15 July 2018
Accepted 31 October 2018

Keywords:
Supplementation
Gestational diabetes
Pregnant women

SUMMARY

Background and aims: This study was designed to assess the effects of combined vitamin D and probiotic supplementation on metabolic status and pregnancy outcomes in women with gestational diabetes (GDM).

Methods: This randomized, double-blind, placebo-controlled clinical trial was performed in 87 women with GDM. Patients were randomly assigned three groups to receive either vitamin D (50,000 IU/every 2 weeks) plus probiotic (8×10^9 CFU/day) (n = 30), probiotic (8×10^9 CFU/day) (n = 29) or placebo (n = 28) for 6 weeks.

Results: Vitamin D and probiotic co-supplementation significantly reduced fasting plasma glucose (β −10.99 mg/dL; 95% CI, −14.26, −7.73; P < 0.001), serum insulin levels (β −1.95 μIU/mL; 95% CI, −3.05, −0.84; P = 0.001) and homeostasis model of assessment-insulin resistance (β −0.76; 95% CI, −1.06, −0.45; P < 0.001), and significantly increased the quantitative insulin sensitivity check index (β 0.01; 95% CI, 0.008, 0.03; P = 0.001) compared with the placebo. In addition, vitamin D and probiotic co-supplementation resulted in a significant reduction in triglycerides (β −37.56 mg/dL; 95% CI, −51.55, −23.56; P < 0.001), VLDL- (β −7.51 mg/dL; 95% CI, −10.31, −4.71; P < 0.001), HDL-/total cholesterol ratio (β −0.52; 95% CI, −0.79, −0.24; P < 0.001), high sensitivity C-reactive protein (β −1.80 mg/L; 95% CI, −2.53, −1.08; P < 0.001) and malondialdehyde (β −0.43 μmol/L; 95% CI, −0.77, −0.09; P = 0.01); also, a significant rise in HDL-cholesterol (β 4.09 mg/dL; 95% CI, 1.11, 7.08; P = 0.008) and total antioxidant capacity (TAC) levels (β 97.77 mmol/L; 95% CI, 52.34, 143.19; P < 0.001) were observed compared with the placebo. Vitamin D and probiotic co-supplementation did not change other metabolic parameters. Vitamin D and probiotic co-supplementation significantly decreased triglycerides (P = 0.02), VLDL-cholesterol (P = 0.02) and hs-CRP (P = 0.01), and significantly increased TAC (P = 0.006) and total glutathione levels (P = 0.04) compared with only probiotic group.

Conclusions: In conclusion, vitamin D and probiotic co-supplementation in women with GDM had beneficial effects on metabolic status.

This trial was registered at www.irct.ir as IRCT2017060-75623N119.

1. Introduction

Gestational diabetes mellitus (GDM) which is defined as glucose intolerance, initially diagnosed during pregnancy accounts for the most common metabolic disorder among pregnant women [1]. There are different modifiable and non-modifiable factors which increases the risk of GDM [2]. Gestational Diabetes Mellitus might be significantly associated with multiple adverse outcomes of pregnancy including cesarean section, macrosomia, large for gestational age, and preterm birth, influencing mother and offspring's health status in later life [3]. Recent evidence has shown that intrauterine

* Corresponding author.
E-mail address: asemi_r@yahoo.com (Z. Asemi).

https://doi.org/10.1016/j.clnu.2018.10.028
0261-5614/

hyperglycemia and decreased antioxidant activity in early life increase the chance of DNA damage in human stem cell, contributing to increased susceptibility to chronic disease in adulthood [4].

Nowadays, it became obvious that vitamin D deficiency is correlated with increased risk of GDM [5] and significant lower of 25-hydroxy vitamin D (25OHD) concentrations were reported in these patients [6,7]. In addition, the relation between low levels of 25OHD and some pregnancy complication is documented in previous studies [8]. On the other hand, rising evidence indicated a change in gut microbiota composition during pregnancy [9] especially in women with GDM [10]. Some studies have suggested that vitamin D treatment may improve insulin resistance and lipid infiltration to the placenta by suppressing mammalian target of rapamycin signaling in women with GDM [11]. Recently, there is evidence to show co-supplementation of vitamin D and probiotic is better work compared with only vitamin D or probiotic supplementation. Probiotics may alter the composition of the microbiota in the gastrointestinal tract through producing antimicrobial substances, which in turn growth and inactivating toxins of pathogenic bacteria [12]. Aside from targeted antimicrobial therapy, probiotics may support colonization resistance through pathways, including increasing mucus production and competition for receptors [13]. On the other hand, probiotic may increase gene expression of vitamin D receptor in the intestinal cells [14]. In a study conducted by Jones et al. [15], supplementation with probiotic *Lactobacillus reuteri* in hypercholesterolemic adults for 9 weeks significantly increased 25OHD concentrations. In addition, vitamin D supplementation may modulate gut microbiota through the regulation of the host immune response [16]. Also, vitamin D supplementation indirectly regulates the microbiome to maintain tolerance in the gastrointestinal tract [17]. Vitamin D administration might be a way to manipulate the composition of the bacterial microbiome [17].

This evidence shows the importance of vitamin D and probiotics co-supplementation. Therefore, we conducted this investigation to evaluate the effects of vitamin D and probiotics co-supplementation on metabolic profiles, biomarkers of inflammation and oxidative stress and pregnancy outcomes in women with GDM.

2. Subjects and methods

2.1. Participants

This study was a randomized, double-blind, placebo-controlled clinical trial, registered in the Iranian registry of clinical trials (http://www.irct.ir:IRCT201706075623N119), performed at a gynecology clinic affiliated to Arak University of Medical Sciences (AUMS), Arak, Iran, between May 2017 to January 2018. Eligible subjects were primigravida, aged 18–40 years (at weeks 24–28 of gestation) who were diagnosed with GDM by a “one-step” 2-h 75-g oral glucose tolerance test based on the American Diabetes Association guidelines [18]. The study was approved by the ethics committee of Arak University of Medical Sciences (AUMS) and voluntarily informed consent was taken from all participants prior to the initiation of the trial. Exclusion criteria included taking vitamin D, probiotic and/or symbiotic supplements during the last 3 months prior to the intervention, insulin therapy during the intervention, pre-eclampsia, eclampsia, hypo and hyperthyroidism, and smokers.

2.2. Study design

Firstly, all women were matched for BMI and age. They were then randomly allocated into three groups to receive either 8×10^9 CFU/g probiotic containing *Lactobacillus acidophilus, Bifidobacterium bifidum, L. reuteri,* and *Lactobacillus fermentum* (each 2×10^9) (n = 29) or 50,000 IU vitamin D3 every 2 weeks plus 8×10^9 CFU/g probiotic containing *L. acidophilus, B. bifidum, L. reuteri,* and *L. fermentum* (each 2×10^9) (n = 30) or placebo (n = 27) for 6 weeks. Although, the duration of intervention was 6 weeks, all women were followed up until the delivery. Vitamin D, probiotic and its placebos (paraffin and starch, respectively) were produced by Zahravi Pharmaceutical Company (Tabriz, Iran), LactoCare®, Zisttakhmir Company (Tehran, Iran) and Barij Essence Pharmaceutical Company (Kashan, Iran), respectively. They were completely identical in terms of their appearance, color, shape, size, smell and taste and packaging. Random assignment was conducted using computer-generated numbers. Randomization and allocation concealment was carried out by a trained staff at the gynecology clinic, about the researchers and participants. All study participants followed the standard pregnancy protocol in Iran, consuming 1000 IU vitamin D3 and 400 μg/day vitamin B9, from the beginning of pregnancy, and 60 mg/day ferrous sulfate, from the second trimester. The compliance rate was assessed by quantifying serum 25(OH) vitamin D levels. Intake of the probiotic, vitamin D3, and placebo capsules was monitored through asking participants to return the medication containers. To increase compliance rate, all patients received brief daily cell phone reminders to take the supplements. All patients were advised to maintain their routine dietary habits without any major changes in lifestyle factors mainly physical activity levels. All patients completed 3-day food records and three physical activity measures as metabolic equivalents (METs) at weeks 0, 3, 6 of the treatment.

2.3. Assessment of anthropometric parameters

A trained staff at the maternity clinic took anthropometric measurements at baseline and 6 weeks following the intervention. Height and weight (Seca, Hamburg, Germany) were measured while the participants wore light clothing and no shoes. BMI was calculated as weight in kg divided by height in meters squared.

2.4. Clinical assessment

Polyhydramnios was diagnosed using the sonographic estimation method at post-intervention. On the basis of this measurement, polyhydramnios was defined as an amniotic fluid index (AFI) in excess of 25 cm. Preterm delivery was defined as delivery occurred at <37 weeks of pregnancy and newborn's macrosomia was defined as birth weight of >4000 g.

2.5. Assessment of biochemical variables

Markers of insulin metabolism were considered as primary outcomes and lipid profiles, biomarkers of inflammation and oxidative stress were considered as secondary outcomes. Ten milliliters fasting blood samples were collected at the beginning and 6-week after the intervention at Arak reference laboratory and centrifuged to separate serum. Serum 25-hydroxyvitamin D concentrations were measured using an ELISA kit (IDS, Boldon, UK) with inter- and intra-assay coefficient of variations (CVs) below 7%. Serum insulin concentrations were quantified by the use of an ELISA kit (DiaMetra, Milano, Italy) with inter- and intra-assay coefficient variances (CVs) of below 5%. The homeostasis model of assessment-insulin resistance (HOMA-IR), and the quantitative insulin sensitivity check index (QUICKI) were determined according to the standard formula [19]. Enzymatic kits (Pars Azmun, Tehran, Iran) were used to quantify fasting plasma glucose (FPG), serum triglycerides, VLDL-, total-, LDL- and HDL-cholesterol concentrations with inter- and intra-assay CVs below 5%. Serum hs-CRP concentrations were determined by commercial

Please cite this article as: Jamilian M et al., The effects of vitamin D and probiotic co-supplementation on glucose homeostasis, inflammation, oxidative stress and pregnancy outcomes in gestational diabetes: A randomized, double-blind, placebo-controlled trial, Clinical Nutrition, https://doi.org/10.1016/j.clnu.2018.10.028

ELISA kit (LDN, Nordhorn, Germany) with inter- and intra-assay CVs below 7%. The plasma nitric oxide (NO) using Griess method [20], total antioxidant capacity (TAC) by the method of ferric reducing antioxidant power developed by Benzie and Strain. [21], total glutathione (GSH) using the method of Beutler et al. [22] and MDA concentrations by the thiobarbituric acid reactive substances spectrophotometric test [23] concentrations were determined by the thiobarbituric acid reactive substances spectrophotometric test with inter- and intra-assay CVs below 5%. Newborns' hyperbilirubinemia was considered when the total serum bilirubin levels were at 15 mg/dL (257 μmol/L) or more among infants who were 25–48 h old, 18 mg/dL (308 μmol/L) in infants who were 49–72 h old, and 20 mg/dL (342 μmol/L) in infants older than 72 h [24].

2.6. Sample size

To calculate the sample size, we used the standard formula suggested for parallel clinical trials by considering type one error (α) of 0.05 and type two error (β) of 0.20 (power = 80%). Based on a previous study [25], we used a standard deviation (SD) of 1.41 and a difference in mean (d) of 1.14, considering HOMA-IR as the key variable. Based on this, we needed 25 persons in each group. Assuming 20% dropouts in each group, the final sample size was determined to be 30 persons per group.

2.7. Statistical analysis

The normality of model residual was tested using the Kolmogorov–Smirnov test. Anthropometric measures and dietary intakes were compared among the three groups, using ANOVA test with Bonferoni post hoc pair-wise comparisons. Multiple linear regression models were used to assess treatment effects on study outcomes after adjusting for baseline values of each biochemical variables. The effect sizes were presented as the mean differences with 95% confidence intervals. Differences in proportions were evaluated by Fisher's exact test. The P-value of<0.05 were considered statistically significant. All statistical analyses used the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, Illinois, USA).

3. Results

Among individuals in the probiotic group, 2 persons [insulin therapy (n = 1) and hospitalization (n = 1)] were excluded. The exclusions in the placebo group were also 3 women [hospitalization (n = 1) and insulin therapy (n = 2)]. Finally, 87 participants [vitamin D plus probiotic (n = 30), probiotic (n = 29) and placebo (n = 28)] completed the trial (Fig. 1). On average, the rate of compliance in our study was high, such that 100% of supplements and placebos were taken throughout the study in three groups.

Mean age, height, weight and BMI at baseline and after the 6-week treatment were not statistically different between treatments and placebo groups (Table 1).

We observed no significant changes in macro- and micronutrients among the three groups (Table 2).

After the 6-week treatment, vitamin D and probiotic co-supplementation significantly reduced FPG (β −10.99 mg/dL; 95% CI, −14.26, −7.73; P < 0.001), serum insulin levels (β −1.95 μIU/mL; 95% CI, −3.05, −0.84; P = 0.001) and HOMA-IR (β −0.76; 95% CI, −1.06, −0.45; P < 0.001), and significantly increased QUICKI (β 0.01; 95% CI, 0.008, 0.03; P = 0.001) compared with the placebo (Table 3). In addition, vitamin D and probiotic co-supplementation resulted in a significant reduction in triglycerides (β −37.56 mg/dL; 95% CI, −51.55, −23.56; P < 0.001), VLDL- (β −7.51 mg/dL; 95% CI, −10.31, −4.71; P < 0.001), HDL-/total cholesterol ratio (β −0.52; 95% CI, −0.79, −0.24; P < 0.001), hs-CRP (β −1.80 mg/L; 95% CI, −2.53, −1.08; P < 0.001) and MDA (β −0.43 μmol/L; 95% CI, −0.77, −0.09; P = 0.01); also, a significant rise in HDL-cholesterol (β 4.09 mg/dL; 95% CI, 1.11, 7.08; P = 0.008) and TAC levels (β 97.77 mmol/L; 95% CI, 52.34, 143.19; P < 0.001) were observed compared with the placebo. Vitamin D and probiotic co-supplementation did not change other metabolic parameters.

Probiotic supplementation resulted in a significant reduction in FPG (β −8.60 mg/dL; 95% CI, −11.96, −5.24; P < 0.001), insulin levels (β −1.34 μIU/mL; 95% CI, −2.46, −0.22; P = 0.01) and HOMA-IR

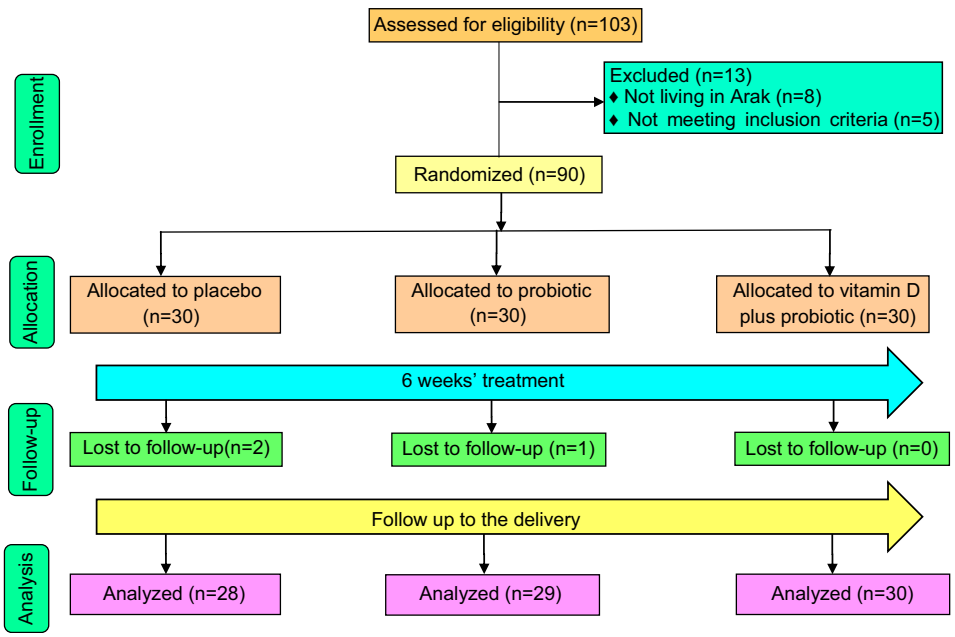

Fig. 1. Summary of patient flow diagram.

Please cite this article as: Jamilian M et al., The effects of vitamin D and probiotic co-supplementation on glucose homeostasis, inflammation, oxidative stress and pregnancy outcomes in gestational diabetes: A randomized, double-blind, placebo-controlled trial, Clinical Nutrition, https://doi.org/10.1016/j.clnu.2018.10.028

Table 1
General characteristics of study participants.[a]

	Placebo group (n = 28)	Probiotic group (n = 29)	Vitamin D plus probiotic group (n = 30)	P[b]
Age (y)	29.9 ± 3.7	31.2 ± 5.9	28.9 ± 6.1	0.28
Height (cm)	162.0 ± 5.8	162.8 ± 4.7	160.8 ± 5.3	0.36
Weight at study baseline (kg)	72.0 ± 7.7	70.0 ± 12.5	71.9 ± 12.1	0.74
Weight at end-of-trial (kg)	73.8 ± 7.7	71.7 ± 12.4	73.6 ± 12.1	0.72
Weight change (kg)	1.8 ± 0.7	1.7 ± 0.6	1.8 ± 0.8	0.86
BMI at study baseline (kg/m^2)	27.5 ± 3.3	26.4 ± 4.2	27.8 ± 4.9	0.37
BMI at end-of-trial (kg/m^2)	28.2 ± 3.3	27.0 ± 4.1	28.5 ± 4.9	0.35
BMI change (kg/m^2)	0.7 ± 0.3	0.6 ± 0.2	0.7 ± 0.3	0.78
MET-h/day at study baseline	25.8 ± 1.1	25.3 ± 1.4	25.2 ± 1.3	0.31
MET-h/day at end-of-trial	25.4 ± 1.1	24.9 ± 1.5	24.8 ± 1.4	0.27
MET-h/day change	−0.4 ± 0.3	−0.4 ± 0.2	−0.4 ± 0.2	0.81

[a] Data are means ± SDs.
[b] Obtained from ANOVA test. METs, metabolic equivalents.

Table 2
Mean dietary intakes of study participants at baseline, weeks 3 and 6 of the study.[a]

	Placebo group (n = 28)	Probiotic group (n = 29)	Vitamin D plus probiotic group (n = 30)	P[b]
Energy (kcal/d)	2201 ± 165	2164 ± 220	2196 ± 192	0.73
Carbohydrates (g/d)	322.8 ± 41.9	313.2 ± 43.8	316.4 ± 42.9	0.69
Protein (g/d)	82.2 ± 16.4	83.2 ± 13.8	85.5 ± 17.3	0.71
Fat (g/d)	80.9 ± 16.9	80.5 ± 10.2	82.1 ± 15.6	0.90
SFA (g/d)	23.0 ± 5.6	24.8 ± 5.0	24.9 ± 6.6	0.37
PUFA (g/d)	27.9 ± 6.4	24.7 ± 5.8	26.5 ± 7.4	0.20
MUFA (g/d)	21.5 ± 6.4	22.0 ± 5.4	21.7 ± 5.8	0.93
Cholesterol (mg/d)	184.2 ± 124.3	233.3 ± 120.3	215.8 ± 133.6	0.33
TDF (g/d)	17.9 ± 4.9	18.5 ± 5.2	18.5 ± 4.1	0.86
Magnesium (mg/d)	273.6 ± 60.8	273.6 ± 69.7	270.7 ± 70.3	0.98
Zinc (mg/d)	9.5 ± 2.8	10.2 ± 2.2	10.4 ± 3.1	0.44
Manganese (mg/d)	2.2 ± 0.8	2.1 ± 0.8	2.2 ± 1.0	0.86
Iron (mg/d)	14.9 ± 3.1	14.6 ± 3.2	14.5 ± 3.2	0.88

MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TDF, total dietary fiber.
[a] Data are means ± SDs.
[b] Obtained from ANOVA test.

(β −0.54; 95% CI, −0.84, −0.23; P < 0.001) compared with the placebo (Table 3). In addition, probiotic supplementation was associated with a significant reduction in triglycerides (β −21.73 mg/dL; 95% CI, −35.78, −7.69; P = 0.003), VLDL-cholesterol (β −4.34 mg/dL; 95% CI, −7.15, −1.53; P = 0.003), hs-CRP (β −1.36 mg/L; 95% CI, −2.07, −0.64; P < 0.001) and MDA levels (β −0.50 μmol/L; 95% CI, −0.85, −0.16; P = 0.005) compared with the placebo. Probiotic supplementation did not affect other metabolic variables. Vitamin D and probiotic co-supplementation significantly decreased triglycerides (β −15.82 mg/dL; 95% CI, −29.66, −1.98; P = 0.02), VLDL-cholesterol (β −3.16 mg/dL; 95% CI, −5.93, −0.39; P = 0.02) and hs-CRP (β −0.32 mg/L; 95% CI, −0.60, −0.05; P = 0.01), and significantly increased TAC (β 63.26 mmol/L; 95% CI, 18.25, 108.26; P = 0.006) and GSH levels (β 53.61 μmol/L; 95% CI, 1.56, 105.67; P = 0.04) compared with only probiotic group.

Co-supplementation with vitamin D and probiotic had a lower incidence of hyperbilirubinemiain newborns (10.0% vs. 13.8% and 35.7%, P = 0.03) and newborns' hospitalization (10.0% vs. 10.3% and 32.1%, P = 0.04) compared with only probiotic and placebo, respectively (Table 4). Co-supplementation with vitamin D and probiotic did not affect other pregnancy outcomes.

4. Discussion

In the current study, we evaluated the effects of a 6-week vitamin D plus probiotic supplementation compared with only probiotic and placebo on metabolic responses and pregnancy outcomes in women with GDM. Our data revealed that vitamin D and probiotic co-supplementation led to significant improvements in glycemic control, lipids concentrations except total- and LDL-cholesterol, and hs-CRP, TAC and MDA levels, incidence of newborn's hyperbilirubinemia and newborns' hospitalization, but did not affect other metabolic profiles and pregnancy outcomes.

4.1. Effects on glycemic control and lipid profiles

GDM women are susceptible to metabolic disorders, including abnormal glucose and lipid metabolism, and inflammation and oxidative damage. In the present study, we found that vitamin D and probiotic co-administration, compared with the placebo, significantly reduced FPG, serum insulin values and HOMA-IR, and significantly increased QUICKI in women with GDM. In line with our findings, probiotic consumption has been reported to reduce insulin resistance in patients with GDM [26] and non-alcoholic fatty liver disease [27]. In addition, our recent study showed that combined vitamin D and probiotic supplementation in diabetic subjects with coronary heart disease for 12 weeks improved insulin concentrations, HOMA-IR and QUICKI, while did not change FPG [28]. In contrast to our findings, Lindsay et al. [29] reported no beneficial effects on glycemic control after probiotic intake in women with GDM. Furthermore, a 4-week supplementation with high-dose vitamin D did not affect insulin secretion and insulin sensitivity [30]. Insulin dysfunction occurs in GDM might both progress to T2DM in later life and leads to neonatal complications [31]. It also can cause adverse long-term maternal outcomes such as increased perinatal morbidity such as macrosomia, birth trauma and pre-eclampsia [32].

Our findings indicated that vitamin D and probiotic co-supplementation, compared with the placebo, significantly decreased serum triglycerides, VLDL-, and total-/HDL-cholesterol

Please cite this article as: Jamilian M et al., The effects of vitamin D and probiotic co-supplementation on glucose homeostasis, inflammation, oxidative stress and pregnancy outcomes in gestational diabetes: A randomized, double-blind, placebo-controlled trial, Clinical Nutrition, https://doi.org/10.1016/j.clnu.2018.10.028

Table 3
Metabolic profiles, biomarkers of inflammation and oxidative stress at study baseline and after the 6-week intervention in patients with gestational diabetes mellitus that received either vitamin D plus probiotic, probiotic supplements or placebo.

Variables	Placebo group (n = 28)		Probiotic group (n = 29)		Vitamin D plus probiotic group (n = 30)		Difference in outcome measures between probiotic and placebo groups[a]		Difference in outcome measures between vitamin D plus probiotic and placebo groups[c]		Difference in outcome measures between vitamin D plus probiotic and probiotic groups[d]	
	Wk0	Wk6	Wk0	Wk6	Wk0	Wk6	β (95% CI)	P[b]	β (95% CI)	P[b]	β (95% CI)	P[b]
25-hydroxyvitamin D (ng/mL)	14.3 ± 4.1	17.7 ± 3.7	12.9 ± 3.2	18.5 ± 3.1	13.4 ± 4.1	35.1 ± 3.9	2.05 (1.19, 2.91)	<0.001	18.21 (17.36, 19.06)	<0.001	16.16 (15.32, 17.00)	<0.001
FPG (mg/dL)	94.1 ± 6.1	93.0 ± 7.9	96.6 ± 3.4	86.5 ± 7.6	95.4 ± 2.2	83.1 ± 5.7	−8.60 (−11.96,−5.24)	<0.001	−10.99 (−14.26,−7.73)	<0.001	−2.39 (−5.62,−0.83)	0.14
Insulin (μIU/mL)	13.6 ± 2.5	13.4 ± 2.9	13.1 ± 7.7	11.7 ± 6.6	12.8 ± 4.6	10.8 ± 5.1	−1.34 (−2.46,−0.22)	0.01	−1.95 (−3.05,−0.84)	0.001	−0.60 (−1.70, 0.48)	0.27
HOMA-IR	3.1 ± 0.6	3.1 ± 0.8	3.1 ± 1.9	2.5 ± 1.5	3.0 ± 1.1	2.2 ± 1.1	−0.54 (−0.84,−0.23)	<0.001	−0.76 (−1.06,−0.45)	<0.001	−0.22 (−0.52, 0.08)	0.15
QUICKI	0.32 ± 0.009	0.32 ± 0.01	0.33 ± 0.03	0.34 ± 0.05	0.32 ± 0.01	0.34 ± 0.03	0.01 (0.00, 0.02)	0.05	0.01 (0.008, 0.03)	0.001	0.008 (−0.003, 0.01)	0.14
Triglycerides (mg/dL)	157.2 ± 39.7	171.9 ± 42.6	160.6 ± 60.5	153.1 ± 60.5	170.5 ± 58.2	145.6 ± 53.4	−21.73 (−35.78,−7.69)	0.003	−37.56 (−51.55,−23.56)	<0.001	−15.82 (−29.66,−1.98)	0.02
VLDL-cholesterol (mg/dL)	31.4 ± 7.9	34.4 ± 8.5	32.1 ± 12.1	30.6 ± 12.1	34.1 ± 11.7	29.1 ± 10.7	−4.34 (−7.15,−1.53)	0.003	−7.51 (−10.31,−4.71)	<0.001	−3.16 (−5.93,−0.39)	0.02
Total cholesterol (mg/dL)	222.6 ± 40.8	225.8 ± 33.0	219.1 ± 36.4	219.6 ± 37.4	221.7 ± 39.1	215.4 ± 46.7	−3.26 (−15.68, 9.16)	0.60	−9.63 (−21.95, 2.67)	0.12	−6.37 (−18.58, 5.83)	0.30
LDL-cholesterol (mg/dL)	135.4 ± 36.6	136.9 ± 33.4	133.1 ± 34.2	134.9 ± 34.1	132.8 ± 35.7	128.4 ± 39.1	−0.09 (−10.98, 10.78)	0.98	−6.30 (−17.10, 4.49)	0.24	−6.20 (−16.90, 4.49)	0.25
HDL-cholesterol (mg/dL)	55.8 ± 14.8	54.5 ± 12.8	53.8 ± 12.0	54.1 ± 10.9	54.9 ± 10.7	57.9 ± 11.3	1.14 (−1.86, 4.16)	0.45	4.09 (1.11, 7.08)	0.008	2.95 (−0.005, 5.91)	0.05
Total-/HDL-cholesterol	4.2 ± 1.2	4.3 ± 1.2	4.2 ± 0.9	4.1 ± 0.8	4.1 ± 1.0	3.8 ± 0.8	−0.19 (−0.47, 0.08)	0.16	−0.52 (−0.79, −0.24)	<0.001	−0.32 (−0.60, −0.05)	0.01
hs-CRP (mg/L)	5.2 ± 2.2	5.7 ± 2.2	5.4 ± 1.9	4.4 ± 1.3	6.2 ± 1.3	4.4 ± 1.3	−1.36 (−2.07,−0.64)	<0.001	−1.80 (−2.53,−1.08)	<0.001	−0.44 (−1.15, 0.26)	0.21
NO (μmol/L)	30.0 ± 4.1	29.8 ± 3.7	32.3 ± 4.5	33.0 ± 5.5	28.5 ± 5.1	29.4 ± 6.7	1.06 (−0.74, 2.87)	0.24	1.00 (−0.77, 2.77)	0.26	−0.06 (−1.90, 1.77)	0.94
TAC (mmol/L)	722.4 ± 140.9	704.2 ± 95.5	723.7 ± 226.7	739.7 ± 224.2	797.6 ± 80.2	860.2 ± 92.1	34.50 (−10.44, 79.46)	0.13	97.77 (52.34, 143.19)	<0.001	63.26 (18.25, 108.26)	0.006
GSH (μmol/L)	577.8 ± 260.3	577.4 ± 246.9	501.3 ± 59.9	511.9 ± 55.6	479.7 ± 100.6	548.8 ± 123.9	−6.06 (−59.92, 47.80)	0.82	47.55 (−6.50, 101.62)	0.08	53.61 (1.56, 105.67)	0.04
MDA (μmol/L)	3.2 ± 0.9	3.4 ± 1.0	2.8 ± 0.6	2.7 ± 0.5	3.3 ± 0.6	3.1 ± 0.7	−0.50 (−0.85,−0.16)	0.005	−0.43 (−0.77,−0.09)	0.01	0.07 (−0.26, 0.42)	0.65

Data are mean ±SDs.
FPG, fasting plasma glucose; GSH, total glutathione; HOMA-IR, homeostasis model of assessment-insulin resistance; HDL-cholesterol, high density lipoprotein-cholesterol; Hs-CRP, high sensitivity C-reactive protein; LDL-cholesterol, low density lipoprotein-cholesterol; MDA, malondialdehyde; NO, nitric oxide; QUICKI, quantitative insulin sensitivity check index; VLDL-cholesterol, very low density lipoprotein-cholesterol; SGA, subjective global assessment; TAC, total antioxidant capacity.

[a] "Outcome measures" refers to the change in values of measures of interest between baseline and week 6. β [difference in the mean outcomes measures between treatment groups (probiotic group = 1 and placebo group = 0)].

[b] Obtained from multiple regression model (adjusted for baseline values of each biochemical variables).

[c] "Outcome measures" refers to the change in values of measures of interest between baseline and week 6. β [difference in the mean outcomes measures between treatment groups (vitamin D plus probiotic group = 1 and placebo group = 0)].

[d] "Outcome measures" refers to the change in values of measures of interest between baseline and week 6. β [difference in the mean outcomes measures between treatment groups (vitamin D plus probiotic group = 1 and probiotic group = 0)].

Table 4
The association of vitamin D plus probiotic supplementation with pregnancy outcomes.

	Placebo group (n = 28)	Probiotic group (n = 29)	Vitamin D+ probiotic group (n = 30)	P[b]
Cesarean section (%)	12 (42.9)	10 (34.5)	7 (23.3)	0.28[a]
Preterm delivery (%)	1 (3.6)	1 (3.4)	0 (0.0)	0.58[a]
Pre-eclampsia (%)	5 (17.9)	3 (10.3)	2 (6.7)	0.39[a]
Polyhydramnios (%)	4 (14.3)	2 (6.9)	2 (6.7)	0.52[a]
Macrosomia>4000 g (%)	5 (17.9)	1 (3.4)	2 (6.7)	0.14[a]
Gestational age (weeks)	38.6 ± 1.1	38.9 ± 2.5	39.3 ± 0.8	0.25
Newborns' weight (g)	3176.4 ± 711.7	3170.7 ± 621.8	3308.3 ± 604.0	0.63
Newborns' length (cm)	49.9 ± 1.7	49.3 ± 3.7	48.7 ± 4.3	0.42
Newborns' head circumference (cm)	35.4 ± 1.5	35.2 ± 2.6	36.2 ± 2.8	0.27
1- min Apgar score	8.8 ± 0.5	8.9 ± 0.3	8.9 ± 0.2	0.28
5- min Apgar score	9.7 ± 0.5	9.9 ± 0.3	9.9 ± 0.2	0.28
Newborns' hyperbilirubinemia (%)	10 (35.7)	4 (13.8)	3 (10.0)	0.03[a]
Newborns' hospitalization (%)	9 (32.1)	3 (10.3)	3 (10.0)	0.04[a]
Newborns' hypoglycemia (%)	4 (14.3)	3 (10.3)	3 (10.0)	0.85[a]

Values are means ± SDs for continuous measures and are number (%) for dichotomous variables.
[a] Obtained from Pearson Chi-square test.
[b] Obtained from ANOVA test.

ratio, and significantly increased HDL-cholesterol, but did not influence total- and LDL-cholesterol levels in patients with GDM. Similarly, probiotic intake in patients with T2DM reduced triglycerides and increased HDL-cholesterol levels [33]. Moreover, vitamin D supplementation to patients with obstructive sleep apnea for 15 weeks increased HDL-cholesterol levels [34]. Increased triglycerides and free fatty acids concentrations in women with GDM are correlated with accelerated fetal growth during pregnancy presented as greater neonatal anthropometric measures [35]. On the other hand, abnormal maternal lipid profiles might be correlated with serious complications such as macrosomia [36], pre-eclampsia and preterm birth [37]. Probiotic consumption influences intestinal bacteria composition and may improve carbohydrate and lipid metabolism by increasing glucagon like peptid-1 secretion, suppression of the toll like reseptor-4 signaling pathway, and modulating the peroxisome proliferator-activated receptor-γ [38]. In addition, vitamin D enhances β-cell function via vitamin D receptors, induce insulin sensitivity and alleviate chronic inflammation which is involved in the developing of insulin resistance [39].

4.2. Effects on biomarkers of inflammation and oxidative stress

Previous evidence has reported elevated circulating inflammatory markers and decreased antioxidant defense in women with GDM [40]. We found that vitamin D and probiotic co-supplementation, compared with the placebo, significantly reduced hs-CRP and MDA, and significantly increased TAC, but did not affect NO and GSH levels. In agreement with our findings, supplementation with vitamin D plus *L. reuteri* to children with allergic asthma for 90 days reduced bronchial inflammation [41]. In addition, taking vitamin D supplementation by diabetic patients with coronary artery disease decreased hs-CRP and MDA levels [42]. However, in adolescents with T1DM and vitamin D deficiency, vitamin D administration did not improve inflammatory markers [43]. Furthermore, taking probiotic product enriched with isoflavones in moderately hypercholesteromic males did not improve CRP levels [44]. One of the main reasons for insulin resistance in GDM might be antioxidant imbalance [45]. Moreover, inflammation can increase the incidence of maternal cardiovascular disease in later life [46]. Oxidative stress and related toxic products can damage biological molecules which increases the susceptibility of offspring to chronic disease [4,47]. Existing experimental studies have shown that antioxidant supplementation may improve insulin sensitivity and decrease GDM complications [48]. Vitamin D and probiotic, with anti-inflammatory and anti-oxidative properties, may be useful to reduce the complications related to GDM. Short chain fatty acid produced by probiotic may exert beneficial effects on inflammation and oxidative stress via modulating the G-protein coupled receptor43 and inducing hydroxyl radical scavenging activity which inhibit lipid peroxidation [49]. In addition, vitamin D reduces gene expression of pro-inflammatory mediators and given the effects on T-regulatory cells, probably modifies the effects of gut microbiome on the immune system and inflammation [50].

4.3. Effects on pregnancy outcomes

In the current study, vitamin D plus probiotic co-supplementation significantly reduced the incidence of newborn's hyperbilirubinemia and hospitalization in comparison to only probiotic and placebo, but did not affect other outcomes. Inconsistent to present data, our prior researches indicated that synbiotic supplementation [51] and calcium plus vitamin D administration [52] in women with GDM decreased the incidence of neonates' hyperbilirubinemia and hospitalization. Decreased incidence of newborn's hyperbilirubinemia and newborns' hospitalization may be due to improved metabolic profiles. Although we agree that further studies are needed to explore such possible mechanisms.

Some limitations need to be taken into account in the interpretation of our findings. Due to funding limitations, we did not characterize the microbiota and thus cannot establish whether probiotic administration over 6 weeks changed its composition. In addition, we did not examine the effects of vitamin D and probiotic supplementation on gene expression related to metabolic profiles.

4.4. Conclusions

In conclusion, vitamin D and probiotic co-supplementation led to significant improvements in glycemic control, lipids concentrations except total- and LDL-cholesterol, and hs-CRP, TAC, GSH and MDA levels, incidence of newborn's hyperbilirubinemia and newborns' hospitalization, but did not affect other metabolic profiles and pregnancy outcomes.

Conflicts of interest

None.

Author contributions

ZA contributed in conception, design, statistical analysis and drafting of the manuscript. MJ and EA contributed in conception, data collection, statistical analysis and manuscript drafting.

Please cite this article as: Jamilian M et al., The effects of vitamin D and probiotic co-supplementation on glucose homeostasis, inflammation, oxidative stress and pregnancy outcomes in gestational diabetes: A randomized, double-blind, placebo-controlled trial, Clinical Nutrition, https://doi.org/10.1016/j.clnu.2018.10.028

Clinical registration

http://www.irct.ir:IRCT201706075623N119.

Acknowledgements

The present study was supported by a grant from the Vice-chancellor for Research, AUMS, and Iran. We are grateful to thank LactoCare®, Zisttakhmir Company, in Tehran that provided probiotic capsules for the present study.

References

[1] American Diabetes Association. Classification and diagnosis of diabetes: standards of medical care in diabetes-2018. Diabetes Care 2018;41:S13–27.
[2] Feng Y, Jiang CD, Chang AM, Shi Y, Gao J, Zhu L, et al. Interactions among insulin resistance, inflammation factors, obesity-related gene polymorphisms, environmental risk factors, and diet in the development of gestational diabetes mellitus. J Matern Fetal Neonatal Med 2018:1–9.
[3] Farrar D, Simmonds M, Bryant M, Sheldon TA, Tuffnell D, Golder S, et al. Hyperglycaemia and risk of adverse perinatal outcomes: systematic review and meta-analysis. BMJ 2016;354:i4694.
[4] Tozour J, Delahaye F, Suzuki M, Praiss A, Zhao Y, Cui L, et al. Intrauterine hyperglycemia is associated with an impaired postnatal response to oxidative damage. Stem Cell Dev 2018;27:683–91.
[5] Lu M, Xu Y, Lv L, Zhang M. Association between vitamin D status and the risk of gestational diabetes mellitus: a meta-analysis. Arch Gynecol Obstet 2016;293:959–66.
[6] Maghbooli Z, Hossein-Nezhad A, Karimi F, Shafaei AR, Larijani B. Correlation between vitamin D3 deficiency and insulin resistance in pregnancy. Diabetes Metab Res Rev 2008;24:27–32.
[7] Burris HH, Rifas-Shiman SL, Kleinman K, Litonjua AA, Huh SY, Rich-Edwards JW, et al. Vitamin D deficiency in pregnancy and gestational diabetes mellitus. Am J Obstet Gynecol 2012;207:182.e1-8.
[8] Weinert LS, Reichelt AJ, Schmitt LR, Boff R, Oppermann ML, Camargo JL, et al. Vitamin D deficiency increases the risk of adverse neonatal outcomes in gestational diabetes. PloS One 2016;11:e0164999.
[9] DiGiulio DB, Callahan BJ, McMurdie PJ, Costello EK, Lyell DJ, Robaczewska A, et al. Temporal and spatial variation of the human microbiota during pregnancy. Proc Natl Acad Sci U S A 2015;112:11060–5.
[10] Gomez-Arango LF, Barrett HL, McIntyre HD, Callaway LK, Morrison M, Dekker Nitert M. Increased systolic and diastolic blood pressure is associated with altered gut microbiota composition and butyrate production in early pregnancy. Hypertension 2016;68:974–81.
[11] Li G, Lin L, Wang YL, Yang H. 1,25(OH)2D3 Protects trophoblasts against insulin resistance and inflammation via suppressing mtor signaling. Reprod Sci 2018. 1933719118766253.
[12] Schoster A, Guardabassi L, Staempfli HR, Abrahams M, Jalali M, Weese JS. The longitudinal effect of a multi-strain probiotic on the intestinal bacterial microbiota of neonatal foals. Equine Vet J 2016;48:689–96.
[13] Collado MC, Meriluoto J, Salminen S. Role of commercial probiotic strains against human pathogen adhesion to intestinal mucus. Lett Appl Microbiol 2007;45:454–60.
[14] Wu S, Yoon S, Zhang YG, Lu R, Xia Y, Wan J, et al. Vitamin D receptor pathway is required for probiotic protection in colitis. Am J Physiol Gastrointest Liver Physiol 2015;309:G341–9.
[15] Jones ML, Martoni CJ, Prakash S. Oral supplementation with probiotic L. reuteri NCIMB 30242 increases mean circulating 25-hydroxyvitamin D: a post hoc analysis of a randomized controlled trial. J Clin Endocrinol Metab 2013;98:2944–51.
[16] Bashir M, Prietl B, Tauschmann M, Mautner SI, Kump PK, Treiber G, et al. Effects of high doses of vitamin D3 on mucosa-associated gut microbiome vary between regions of the human gastrointestinal tract. Eur J Nutr 2016;55:1479–89.
[17] Ooi JH, Li Y, Rogers CJ, Cantorna MT. Vitamin D regulates the gut microbiome and protects mice from dextran sodium sulfate-induced colitis. J Nutr 2013;143:1679–86.
[18] American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2014;37(Suppl 1):S81–90.
[19] Pisprasert V, Ingram KH, Lopez-Davila MF, Munoz AJ, Garvey WT. Limitations in the use of indices using glucose and insulin levels to predict insulin sensitivity: impact of race and gender and superiority of the indices derived from oral glucose tolerance test in African Americans. Diabetes Care 2013;36:845–53.
[20] Tatsch E, Bochi GV, Pereira Rda S, Kober H, Agertt VA, de Campos MM, et al. A simple and inexpensive automated technique for measurement of serum nitrite/nitrate. Clin Biochem 2011;44:348–50.
[21] Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996;239:70–6.
[22] Beutler E, Gelbart T. Plasma glutathione in health and in patients with malignant disease. J Lab Clin Med 1985;105:581–4.
[23] Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic Biol Med 1990;9:515–40.
[24] Porter ML, Dennis BL. Hyperbilirubinemia in the term newborn. Am Fam Physician 2002;65:599–606.
[25] Asemi Z, Hashemi T, Karamali M, Samimi M, Esmaillzadeh A. Effects of vitamin D supplementation on glucose metabolism, lipid concentrations, inflammation, and oxidative stress in gestational diabetes: a double-blind randomized controlled clinical trial. Am J Clin Nutr 2013;98:1425–32.
[26] Taylor BL, Woodfall GE, Sheedy KE, O'Riley ML, Rainbow KA, Bramwell EL, et al. Effect of probiotics on metabolic outcomes in pregnant women with gestational diabetes: a systematic review and meta-analysis of randomized controlled trials. Nutrients 2017;9.
[27] Ma YY, Li L, Yu CH, Shen Z, Chen LH, Li YM. Effects of probiotics on nonalcoholic fatty liver disease: a meta-analysis. World J Gastroenterol 2013;19:6911–8.
[28] Raygan F, Ostadmohammadi V, Bahmani F, Asemi Z. The effects of vitamin D and probiotic co-supplementation on mental health parameters and metabolic status in type 2 diabetic patients with coronary heart disease: a randomized, double-blind, placebo-controlled trial. Prog Neuro-Psychopharmacol Biol Psychiatry 2018;84:50–5.
[29] Lindsay KL, Brennan L, Kennelly MA, Maguire OC, Smith T, Curran S, et al. Impact of probiotics in women with gestational diabetes mellitus on metabolic health: a randomized controlled trial. Am J Obstet Gynecol 2015;212:496.e1-11.
[30] Gulseth HL, Wium C, Angel K, Eriksen EF, Birkeland KI. Effects of vitamin D supplementation on insulin sensitivity and insulin secretion in subjects with type 2 diabetes and vitamin d deficiency: a randomized controlled trial. Diabetes Care 2017;40:872–8.
[31] Landon MB, Mele L, Spong CY, Carpenter MW, Ramin SM, Casey B, et al. The relationship between maternal glycemia and perinatal outcome. Obstet Gynecol 2011;117:218–24.
[32] Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, et al. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med 2008;358:1991–2002.
[33] He J, Zhang F, Han Y. Effect of probiotics on lipid profiles and blood pressure in patients with type 2 diabetes: a meta-analysis of RCTs. Medicine (Baltimore) 2017;96:e9166.
[34] Kerley CP, Hutchinson K, Bramham J, McGowan A, Faul J, Cormican L. Vitamin D improves selected metabolic parameters but not neuropsychological or quality of life indices in osa: a pilot study. J Clin Sleep Med 2017;13:19–26.
[35] Schaefer-Graf UM, Graf K, Kulbacka I, Kjos SL, Dudenhausen J, Vetter K, et al. Maternal lipids as strong determinants of fetal environment and growth in pregnancies with gestational diabetes mellitus. Diabetes Care 2008;31:1858–63.
[36] Kitajima M, Oka S, Yasuhi I, Fukuda M, Rii Y, Ishimaru T. Maternal serum triglyceride at 24–32 weeks' gestation and newborn weight in nondiabetic women with positive diabetic screens. Obstet Gynecol 2001;97:776–80.
[37] Vrijkotte TG, Krukziener N, Hutten BA, Vollebregt KC, van Eijsden M, Twickler MB. Maternal lipid profile during early pregnancy and pregnancy complications and outcomes: the ABCD study. J Clin Endocrinol Metab 2012;97:3917–25.
[38] den Besten G, Bleeker A, Gerding A, van Eunen K, Havinga R, van Dijk TH, et al. Short-chain fatty acids protect against high-fat diet-induced obesity via a ppargamma-dependent switch from lipogenesis to fat oxidation. Diabetes 2015;64:2398–408.
[39] Chagas CE, Borges MC, Martini LA, Rogero MM. Focus on vitamin D, inflammation and type 2 diabetes. Nutrients 2012;4:52–67.
[40] Lekva T, Norwitz ER, Aukrust P, Ueland T. Impact of systemic inflammation on the progression of gestational diabetes mellitus. Curr Diab Rep 2016;16:26.
[41] Miraglia Del Giudice M, Maiello N, Allegorico A, Iavarazzo L, Capasso M, Capristo C, et al. *Lactobacillus reuteri* DSM 17938 plus vitamin D3 as ancillary treatment in allergic children with asthma. Ann Allergy Asthma Immunol 2016;117:710–2.
[42] Farrokhian A, Raygan F, Bahmani F, Talari HR, Esfandiari R, Esmaillzadeh A, et al. Long-term vitamin D supplementation affects metabolic status in vitamin d-deficient type 2 diabetic patients with coronary artery disease. J Nutr 2017;147:384–9.
[43] Shih EM, Mittelman S, Pitukcheewanont P, Azen CG, Monzavi R. Effects of vitamin D repletion on glycemic control and inflammatory cytokines in adolescents with type 1 diabetes. Pediatr Diabetes 2016;17:36–43.
[44] Cavallini DC, Manzoni MS, Bedani R, Roselino MN, Celiberto LS, Vendramini RC, et al. Probiotic soy product supplemented with isoflavones improves the lipid profile of moderately hypercholesterolemic men: a randomized controlled trial. Nutrients 2016;8.
[45] Houstis N, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature 2006;440:944–8.
[46] Lekva T, Bollerslev J, Norwitz ER, Aukrust P, Henriksen T, Ueland T. Aortic stiffness and cardiovascular risk in women with previous gestational diabetes mellitus. PloS One 2015;10:e0136892.
[47] Moreli JB, Santos JH, Lorenzon-Ojea AR, Correa-Silva S, Fortunato RS, Rocha CR, et al. Hyperglycemia differentially affects maternal and fetal dna integrity and dna damage response. Int J Biol Sci 2016;12:466–77.

Please cite this article as: Jamilian M et al., The effects of vitamin D and probiotic co-supplementation on glucose homeostasis, inflammation, oxidative stress and pregnancy outcomes in gestational diabetes: A randomized, double-blind, placebo-controlled trial, Clinical Nutrition, https://doi.org/10.1016/j.clnu.2018.10.028

[48] Panigrahy SK, Bhatt R, Kumar A. Reactive oxygen species: sources, consequences and targeted therapy in type 2 diabetes. J Drug Target 2017;25:93–101.
[49] Shah C, Mokashe N, Mishra V. Preparation, characterization and in vitro antioxidative potential of synbiotic fermented dairy products. J Food Sci Technol 2016;53:1984–92.
[50] Wasilewski A, Zielinska M, Storr M, Fichna J. Beneficial effects of probiotics, prebiotics, synbiotics, and psychobiotics in inflammatory bowel disease. Inflamm Bowel Dis 2015;21:1674–82.
[51] Karamali M, Nasiri N, Taghavi Shavazi N, Jamilian M, Bahmani F, Tajabadi-Ebrahimi M, et al. The effects of synbiotic supplementation on pregnancy outcomes in gestational diabetes. Probiotics Antimicrob Proteins 2018;10:496–503.
[52] Karamali M, Asemi Z, Ahmadi-Dastjerdi M, Esmaillzadeh A. Calcium plus vitamin D supplementation affects pregnancy outcomes in gestational diabetes: randomized, double-blind, placebo-controlled trial. Public Health Nutr 2016;19:156–63.

Please cite this article as: Jamilian M et al., The effects of vitamin D and probiotic co-supplementation on glucose homeostasis, inflammation, oxidative stress and pregnancy outcomes in gestational diabetes: A randomized, double-blind, placebo-controlled trial, Clinical Nutrition, https://doi.org/10.1016/j.clnu.2018.10.028